

- 9:00pm Monday through Friday * * 7:30am - 5:00pm Saturday, Sunday, Holidays *
* * * * * APS is unavailable Thanksgiving Day, Christmas * *
Day, * * and New Year's Day. * *
* * * * * FILE 'USPAT' ENTERED AT
09:06:49 ON 28 SEP 95

***** * WELCOME TO TH
E * * U. S. PATENT TEXT FILE * *

=> s respiratory(w)syncytial
11174 RESPIRATORY
366 SYNCYTIAL
L1 289 RESPIRATORY(W)SYNCYTIAL

=> s l1 and (ion(w)exchange or gel(w)filtration)
129077 ION
123430 EXCHANGE
34850 ION(W)EXCHANGE
121548 GEL
115654 FILTRATION
5728 GEL(W)FILTRATION
L2 70 L1 AND (ION(W)EXCHANGE OR GEL(W)FILTRATION)
=> s respiratory(w)syncytial/ti or respiratory(w)syncytial/ab 11174 RESPIRATORY
11 SYNCYTIAL/TI
11 RESPIRATORY(W)SYNCYTIAL/TI
11174 RESPIRATORY
18 SYNCYTIAL/AB
17 RESPIRATORY(W)SYNCYTIAL/AB
L3 17 RESPIRATORY(W)SYNCYTIAL/TI OR RESPIRATORY(W)SYNCYTIAL/AB
=> s l3 and (ion(w)exchange or gel(w)filtration)
129077 ION
123430 EXCHANGE

34850 ION(W)EXCHANGE
121548 GEL
115654 FILTRATION
5728 GEL(W)FILTRATION
L4 7 L3 AND (ION(W)EXCHANGE OR GEL(W)FILTRATION)
=> d l4 1-7 cit kwic

e 351:DERWENT WPI 1981-1995/UD=9536;UA=9530;UM=9525
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Set Items Description

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?s respiratory(w)syncytial

4334 RESPIRATORY

118 SYNCYTIAL

S1 113 RESPIRATORY(W)SYNCYTIAL

?s s1 and ion(w)exchange

113 S1

81574 ION

90351 EXCHANGE

13464 ION(W)EXCHANGE

S2 0 S1 AND ION(W)EXCHANGE

?s s1 and gel(w)filtration

113 S1

38750 GEL

26139 FILTRATION

1668 GEL(W)FILTRATION

S3 1 S1 AND GEL(W)FILTRATION

?t s3/6/1

3/6/1

009981430 WPI Acc No: 94-249144/30

XRAM Acc No: C94-113344

Refolding FG glyco-protein from human ***respiratory*** ***syncytial*** virus - by treatment with guanidine and detergent, giving pure, immunologically active product for use in vaccines

?t s3/7/1

3/7/1

DIALOG(R)File 351:DERWENT WPI

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009981430 WPI Acc No: 94-249144/30

XRAM Acc No: C94-113344

Refolding FG glyco-protein from human ***respiratory*** ***syncytial*** virus - by treatment with guanidine and detergent, giving pure, immunologically active product for use in vaccines

Patent Assignee: (UPJO) UPJOHN CO

Author (Inventor): GARLICK R L; LYLE S B; WATHEN M; WELLS P A Number of Patents: 002

Number of Countries: 046

Patent Family:

| CC Number | Kind | Date | Week |
|------------|------|--------|--------------|
| WO 9415968 | A1 | 940721 | 9430 (Basic) |
| AU 9459552 | A | 940815 | 9442 |

Priority Data (CC No Date): US 1874 (930108)

Applications (CC,No,Date): AU 9459552 (931229); WO 93US12373 (931229) Language: English

EP and/or WO Cited Patents: 01Jnl.Ref; EP 433225; WO 8905823 Designated States

(National): AT; AU; BB; BG; BR; BY; CA; CH; CZ; DE; DK; ES; FI; GB; HU; JP ; KP; KR; KZ; LK; LU; LV; MG; MN; MW; NL; NO; NZ; PL; PT; RO; RU; SD; SE; SK; UA; US; UZ; VN

(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LU; MC; NL; OA; PT ; SE

Filing Details: AU9459552 Based on WO 9415968

Abstract (Basic): WO 9415968 A

Proper configuration of an FG glycoprotein (I, a chimeric protein contg. glycoproteins from human ***respiratory*** ***syncytial*** virus RSV), is restored by treating a soln. of denatured (I) with (a) enough guanidine to give concn. 5-8 M, (b) a buffer of pK 5-10, (c) a non-ionic or zwitterionic detergent, and (d) basic soln. to give pH 5-10. The guanidine is then removed by dialysis, diafiltration or ***gel*** ***filtration***.

Also claimed is a method of purificn. of (I) to better than 90% by reverse phase chromatography then refolding as above. (I) produced by these processes are themselves claimed.

USE/ADVANTAGE - (I) is useful in vaccines to protect against RSV. The refolded (I) is pure (pref. better than 95%) and immunologically active. Dwg.0/0

Derwent Class: A96; B04;

Int Pat Class: C07K-003/08; C07K-015/00

Derwent Registry Numbers: 0956-S

?b 155,5,73

SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1966-1995/Nov W2

(c) format only 1995 Knight-Ridder Info

File 5:BIOSIS PREVIEWS(R) 1969-1995/Sep W4

(c) 1995 BIOSIS

*File 5: s (Meeting()Abstract) or abstracts/DE for 1994 + conference records

File 73:EMBASE 1974-1995/Iss 38

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Set Items Description

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?s respiratory(w)syncytial

601651 RESPIRATORY

12581 SYNCYTIAL

S1 8990 RESPIRATORY(W)SYNCYTIAL

?s s1 and ion(w)exchange

8990 S1

399234 ION

257481 EXCHANGE

67698 ION(W)EXCHANGE

S2 10 S1 AND ION(W)EXCHANGE

?rd

...completed examining records

S3 5 RD (unique items)

?t s3/7/1-5

3/7/1 (Item 1 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

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09107721 95037721

Purification of a recombinant human ***respiratory*** ***syncytial*** virus chimeric glycoprotein using reversed-phase chromatography and protein refolding in guanidine hydrochloride.

Wells PA; Garlick RL; Lyle SB; Tuls JL; Poorman RA; Brideau RJ; Wathen MW Upjohn Company, Kalamazoo, Michigan 49001.

Protein Expr Purif (UNITED STATES) Aug 1994, 5 (4) p391-401, ISSN 1046-5928 Journal Code: BJV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

FG glycoprotein is a recombinant chimeric protein consisting of the extracellular portions of human ***respiratory*** ***syncytial*** virus (RSV) F and G glycoproteins. In theory, highly purified FG glycoprotein may be effective as a RSV vaccine. Recombinant FG glycoprotein was expressed using the baculovirus/insect cell system. FG glycoprotein was isolated from cell culture supernatants using S Sepharose ***ion***-***exchange*** chromatography, Cu(2+)-immobilized metal affinity chromatography, preparative reversed-phase high-performance liquid chromatography, denaturation with 6 M guanidine hydrochloride, and protein refolding in Tween 80 detergent. The purified FG glycoprotein was concentrated on a S Sepharose column and exchanged into an appropriate buffer for vaccine formulation. Five batches of FG glycoprotein with protein purity of 92-99% were produced using this purification process. FG glycoprotein produced using reversed-phase chromatography and protein refolding was

compared with nondenatured FG glycoprotein using a panel of 14 monoclonal antibodies directed against conformational and linear epitopes on RSV F and G glycoproteins. The results of these studies indicated that refolded FG glycoprotein had the same three-dimensional structure as nondenatured FG glycoprotein.

3/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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08250338 92388338

Active ***respiratory*** ***syncytial*** virus purified by ***ion***- ***exchange*** chromatography: characterization of binding and elution requirements.

Downing LA; Bernstein JM; Walter A

Department of Physiology and Biophysics, Wright State University, Dayton, OH.

J Virol Methods (NETHERLANDS) Aug 1992, 38 (2) p215-28, ISSN 0166-0934 Journal Code: HQR

Contract/Grant No.: S15-DK-42204, DK, NIDDK

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Two viruses, ***respiratory*** ***syncytial*** virus (RSV) and vesicular stomatitis virus (VSV) were used to evaluate viral purification by an affinity resin column (Matrex Cellufine Sulfate (MCS); Amicon Division, WR Grace & Co.). Viable RSV was purified significantly from crude cell lysate by a single pass through a column containing the anionic MCS resin. Most cell protein and albumin eluted from the MCS resin with phosphate buffered saline (PBS) but RSV eluted at high ionic strength, i.e., greater than or equal to 0.6 M NaCl. Further purification was possible by sucrose step gradient centrifugation. The RSV prepared by column purification or by column plus sucrose gradient separation was both intact and infective. RSV and pure samples of VSV were used to optimize ionic strength and salts for elution from the MCS column: 0.8 M NaCl removed most of the viral protein. The capacity of the MCS gel for RSV or VSV was found to be about 0.6-0.8 mg viral protein per ml of hydrated resin. Detergent-solubilized viral membrane proteins bound to the MCS resin in 0.145 M NaCl and eluted with higher salt concentrations. Thus, this resin also may be a useful aid for relatively gentle purification of these proteins.

3/7/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07432230 90339230

High-dose, short-duration ribavirin aerosol therapy in children with suspected ***respiratory*** ***syncytial*** virus infection. Englund JA; Piedra PA; Jefferson LS; Wilson SZ; Taber LH; Gilbert BE
Department of Microbiology and Immunology, Baylor College of Medicine, Houston, Texas 77030.

J Pediatr (UNITED STATES) Aug 1990, 117 (2 Pt 1) p313-20, ISSN 0022-3476 Journal Code: JLZ

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Nine children (aged 6 weeks to 7 years) with suspected ***respiratory*** ***syncytial*** virus infection received aerosol treatment with ribavirin, 60 mg/ml for 2-hour periods three times daily for up to 5 days. Five children received treatment via an endotracheal tube and four via an oxygen hood. Blood samples (3 to 17 per patient) and respiratory secretions (4 to 23 per patient) were assayed for ribavirin with reverse-phase high-performance liquid chromatography. Ribavirin triphosphate in erythrocytes was determined by ***ion***-***exchange*** high-performance liquid chromatography. The mean (+/- SD) peak ribavirin level after the first dose was 1725 +/- 2179 mumol/L in secretions and 3.8 +/- 2.6 mumol/L in plasma. Ribavirin in the secretions was rapidly cleared, with a mean (+/- SD), half-life of 1.9 +/- 0.8 hours. Plasma ribavirin increased with treatments to reach a steady state of 5 to 10 mumol/L. Mean peak

ribavirin triphosphate levels were 15- to 300-fold higher than plasma ribavirin levels by the end of therapy. More than 98% reduction of viral load without the emergence of resistant virus was noted on day 3 of therapy. High-dose treatment was compatible with the aerosol equipment routinely used (small-particle aerosol generator, model 2-6000) for ribavirin administration and with ventilators. High-dose, short-duration ribavirin therapy was well tolerated by all patients, permitted easier accessibility for patient care, and may result in less environmental exposure of health care workers.

3/7/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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07025473 89327473

Comparison of monoclonal antibody time-resolved fluoroimmunoassay with monoclonal antibody capture-biotinylated detector enzyme immunoassay for ***respiratory*** ***syncytial*** virus and parainfluenza virus antigen detection.

Hierholzer JC; Bingham PG; Coombs RA; Johansson KH; Anderson LJ; Halonen PE

Division of Viral Diseases, Centers for Disease Control, Atlanta, Georgia 30333.

J Clin Microbiol (UNITED STATES) Jun 1989, 27 (6) p1243-9, ISSN 0095-1137 Journal Code: HSH

Languages: ENGLISH

Document type: JOURNAL ARTICLE

An all-monoclonal antibody, time-resolved fluoroimmunoassay was compared with several enzyme immunoassays for the detection of ***respiratory*** ***syncytial*** virus and parainfluenza virus type 1, 2, and 3 antigens in clinical specimens. The most sensitive enzyme immunoassay for parainfluenza virus type 1 was an all-monoclonal antibody assay with biotin-labeled detector antibody and streptavidin-peroxidase conjugate, but for ***respiratory*** ***syncytial*** virus and parainfluenza virus types 2 and 3 the most sensitive assay was a polyclonal antibody assay with horse capture antibodies and bovine or rabbit detector antibodies with anti-species peroxidase. All tests were evaluated with nasopharyngeal aspirate specimens from respiratory illnesses and with cell culture harvests of multiple strains of each virus isolated over many years. The time-resolved fluoroimmunoassay detected ***respiratory*** ***syncytial*** virus antigen in 92% of the specimens positive by culture, which was a decidedly higher sensitivity than either the monoclonal or polyclonal antibody enzyme immunoassay format (62 and 76%, respectively). For the parainfluenza viruses the time-resolved fluoroimmunoassay detected type-specific antigen in 94 to 100% of culture-positive specimens and again was more sensitive than the all-monoclonal antibody enzyme immunoassays (75 to 89%) or all-polyclonal antibody enzyme immunoassays (66 to 95%). Combined with results from a previously reported adenovirus time-resolved fluoroimmunoassay, these tests identified respiratory antigens in large numbers of clinical specimens.

3/7/5 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
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9282508 EMBASE No: 94234992

Respiratory ***syncytial*** virus-specific cell-mediated immune responses after vaccination with a purified fusion protein subunit vaccine Welliver R.C.; Tristram D.A.; Batt K.; Sun M.; Hogerman D.; Hildreth S. Children's Hospital, 219 Bryant St., Buffalo, NY 14222 USA J. INFECT. DIS. (USA) , 1994, 170/2 (425-428) CODEN: JIDIA ISSN: 0022-1899

LANGUAGES: English SUMMARY LANGUAGES: English

Vaccination with a ***respiratory*** ***syncytial*** virus (RSV) fusion protein subunit vaccine (PFP-2) was done to determine if this vaccine induced evidence of cell-mediated immunity to RSV and if cell-mediated immunity prevented RSV reinfection. Healthy children 12-18 months old received 50 microg of PFP-2 or a saline control. Lymphocyte transformation (LTF) responses were determined before and 1

and 6 months after vaccination. PFP-2 induced positive LTF responses in 5 (83%) of 6 subjects whose prevaccination samples lacked evidence of cell-mediated immunity. Positive LTF responses in prevaccination samples were not boosted but were more persistent in vaccinees than in controls. Positive LTF responses were not associated with protection against subsequent infection. Immunization with PFP-2 induces correlates of cell-mediated immunity, but this immunity does not appear to be a critical component of protection.
?s s1 and gel(w)filtration

8990 S1
426254 GEL
163072 FILTRATION
72688 GEL(W)FILTRATION
S4 6 S1 AND GEL(W)FILTRATION
?rd

...completed examining records
S5 2 RD (unique items)
?t s5/7/1-2

5/7/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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06206805 87180805

Neutralizing activity in human milk fractions against ***respiratory*** ***syncytial*** virus.

Laegreid A; Kolsto Otnaess AB; Orstavik I; Carlsen KH

Acta Paediatr Scand (SWEDEN) Sep 1986, 75 (5) p696-701, ISSN 0001-656X Journal Code: 1LV
Languages: ENGLISH

Document type: JOURNAL ARTICLE

Neutralizing activity against ***respiratory*** ***syncytial*** virus (RSV) was measured in milk samples from 17 healthy women whose infants had an acute infection with ***respiratory*** ***syncytial*** virus (RSV) and from 27 women with healthy infants. All milk samples were obtained 2-8 months post partum. Neutralizing activity was detected in 36 samples. No major difference in neutralizing titers was observed between the two groups, and the titers were low. RSV-specific IgA was found in two samples, and RSV-specific IgG in one sample. RSV-specific IgM was not detected. In ***gel*** ***filtration*** studies, the neutralizing activity was eluted with an apparent molecular weight above 400,000. The neutralizing activity remained after removal of IgA by affinity chromatography. These findings suggest that both immunoglobulin and non-immunoglobulin components in human milk can neutralize RSV.

5/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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05841501 86142501

Interleukin 1 and interleukin 1 inhibitor production by human macrophages exposed to influenza virus or ***respiratory*** ***syncytial*** virus. ***Respiratory*** ***syncytial*** virus is a potent inducer of inhibitor activity.

Roberts NJ Jr; Prill AH; Mann TN

J Exp Med (UNITED STATES) Mar 1 1986, 163 (3) p511-9, ISSN 0022-1007 Journal Code: I2V

Contract/Grant No.: AI 15547; HL 07496

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Respiratory viral infections are commonly associated with altered immune responses, such as proliferative

responses to mitogens and antigens. To examine potential mechanisms, we examined production of IL-1 and IL-1 inhibitors by purified human peripheral blood-derived macrophages exposed to influenza virus or ***respiratory*** ***syncytial*** virus (RSV). IL-1 and IL-1 inhibitor activities in supernatant fluids from macrophages exposed to the viruses 24 h previously were measured using the standard mouse thymocyte comitogen assay. Crude fluids from macrophages exposed to influenza virus contained substantial IL-1 activity, whereas crude fluids from macrophages exposed to RSV contained marked IL-1 inhibitor activity. Assays with ***gel*** ***filtration***-separated fractions revealed that both influenza virus and RSV induced production of both IL-1 and IL-1 inhibitors. Neither IL-2 nor IL-2 inhibitor activities were detected. Thus, effects of human macrophage-derived factors on thymocyte proliferation, or potentially on human lymphocyte proliferation, may reflect the total or net activity of multiple composite factors, the balance of which varies according to the challenge. The data raise the possibility that marked production of IL-1 inhibitor activity in response to RSV plays a role in the clinical recurrence of RSV infection despite the absence of clear evidence for antigenic shift or drift of the virus.

?logoff hold